VALIDATION OF MRI PATTERNS OF GLIOBLASTOMA MULTIFORME TO PREDICT MGMT METHYLATION

DEGREE FINAL PROJECT

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ABBREVIATIONS

ADC	Apparent diffusion coefficient
BBB	Blood-brain barrier
BOLD	Blood oxygenation level dependent
CBF	Cerebral blood flow
CBV	Cerebral blood flow
CE	Contrast-enhanced
DSC	Dynamic susceptibility contrast
DWI	Diffusion weighted image
FLAIR	Fluid-attenuated inversion recovery
GBM	Glioblastoma multiforme
GRE	Gradient Echo
GTR	Gross-total resection
Gy	Gray
HGG	High grade glioma
ICO	Institut Català d'oncologia
IDH	Isocitrate dehydrogenase
KPS	Karnofsky performance scale
LGG	Low grade glioma
MGMT	O6-methylguanine-DNA methyltransferase
MMS	Mini Mental scale
MRI	Magnetic resonance imaging
OS	Overall survival
PACS	Picture Archiving and Communication System
PFS	Progression-free survival
PWI	Perfusion-weighted imaging
RT	Radiotherapy
TMZ	Temozolomide
WI	Weighted-image
GBMP	Primary glioblastoma multiforme
GBMS	Secondary glioblastoma multiforme

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1. ABSTRACT

Background: Glioblastoma Multiforme (GBM) is the most frequent and deadliest of malignant brain tumors with a mean overall survival of 24% at first year. In the last decade magnetic resonance imaging (MRI) has become an essential tool for diagnosis, but is still not perfect as the final diagnostic is based on the histopathological analysis. In the last years numerous studies have observed that radiological characteristics may reflect the biological features of GBM and may be associated with genetic aberrations and molecular alterations that occur in tumorigenesis. Radiological patters have been also related to prognosis and patient outcomes. In that way, is logical to think that there is a link between some radiological patterns, molecular properties and, because of that, prognosis.

MRI could complement the use of biopsy as a diagnostic instrument in the molecular diagnosis of glioblastoma, and the best way to predict patient's prognosis and to give realistic expectation to the patients.

Objective: To validate MRI patterns of Glioblastoma Multiforme to predict the IDH and MGMT status.

Design: A multicentric, observational, prospective cohort with 3 years of follow-up. Three third level hospitals of Institut Català d'Oncologia (ICO): Duran i Renyals hospital, Germans Trias i Pujol hospital, and Dr. Josep Trueta hospital will participate in this project.

Participants: people older than 18 years old that have been diagnosed of GBM in the 2 years of duration of the sample collection, and had no documented history of previous brain tumors.

Methods: Information of 240 patients with confirmed GBM will be analysed, including pre and postoperative MRI and the clinical features. The data will be obtained mainly by the informatic date base, with the patient's medical histories, that links the hospitals. For the statistical analysis we will use a chi-squared test, a T-student or Mann-Withney's U depending on the symmetry of the variables. The progression-free survival (PFS) and overall survival (OS) will be summarized with the Kapplan-Meier survival curve estimator and then asses it with the Log-rank test. A multivariate analysis will be also done adjusting using logistic regressions and the Cox regression.

Keywords: Glioblastoma, IDH status, MGMT promoter methylation, Radiogenomics, MRI biomarker

2. INTRODUCTION

2.1. Definition of Glioblastoma

GBM is a grade IV glioma according to the WHO classification of gliomas (1). It is characterized for having grade III typical lesions, lesions that have cellular atypia together with anaplasia and mitotic activity; they further present microvascular proliferation and/or necrosis. GBM is the only representative tumor of grade IV gliomas (2,3).

2.2 Epidemiology

GBM is the most frequent type of primary malignant brain tumor and the most frequent type of Astrocytoma. It has a 5-year survival below 5%.

The incidence in North America is of 3-4 per 100.000 inhabitants/year, although countries such as South Corea republic have an incidence of 0,59 per 100.000 inhabitants/year. GBM rate is 2.5 times higher in European Americans than in African Americans and more common in non-Hispanics than in Hispanics (4,5). Males are approximately 1.7 times more often affected than females (6).

In Girona, Catalonia, the incidence is 4.17 (CI 95% 3.80-4.57) cases per 100.000 inhabitants/year, showing a bit more predominance in men. The mean age of presentation is of 64 years old. It presents an overall survival of 24% at 1st year (CI 95% 20.4-28.3), and of 3.3% at 5 years (CI 95% 2.0-5.6). And a relative survival of 24% (CI 95% 20.8-28.7) at first year and of 3.7% at 5 years (CI 95% 2.3-6.2) (7).

Risk factors

Factors associated in GBM risk are prior low grade astrocytoma, decreased susceptibility to allergy, immune factors and immune genes, and some nucleotide polymorphisms detected by genome-wide association (8).

There is no substantial evidence of GBM association with lifestyle characteristics such as cigarette smoking, alcohol consumption, drug use or dietary exposure to nitrous compounds (8).

Tumor location

Tumor location is a key parameter in the care of patients with GBM because it correlates with clinical presentation, histomolecular characteristics, surgical management, delivery of subsequent oncologic treatments and, therefore, outcomes.

Superficial location distant from the eloquent areas is associated with a preserved functional status at diagnosis, a subtotal or gross total surgical resection and prolonged PFS and overall survival OS.

Deep tumor location and a brain eloquent area location are associated with an impaired functional status at diagnosis, major neurologic deficit, complicated surgical access in which many times prevents complete resection and shortened PFS and for OS (9).

Prognostic factors

Although various treatment strategies have been used, GBM patients typically still have a poor prognosis with median PFS and OS of 6.9 months and 14.7 months, respectively and a median survival of only 3 months in untreated patients (8). Its clinical outcome varies substantially, with some patients succumbing to progressive disease within weeks while others survive for more than 5 years (see Table 2, Annex 1) (10–12).

Previously investigated prognostic factors for GBM are: clinical characteristics, including patient age, Karnofsky Performance score (KPS), the extent of resection, the resecability of the tumor, gross total resection (GTR), its location, , size, multifocality, as well as advanced age (>70 years), comorbidities and molecular features (8). GTR was associated with improved survival as compared to subtotal resection; obtaining a post-operative T1CE MRI within 24–48 hours of surgery (10–12). Isocitrate dehydrogenase (IDH) genes status and O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation are, among others, important molecular characteristics in this type of tumor (OS with only an IDH1 mutation is shorter than for patients with both IDH1 mutations and MGMT methylation; see Figure 12, Annex 2).

Metastases

The lack of lymphatic system, the high dural density of intracranial veins and the lack of stroma that can feed GBM cell's outside central nervous system are the principal mechanisms that prevent the metastatic dissemination (13,14).

The most common metastatic locations (0,4-2%) have been described in lung and pleura (60%), lymphatic ganglia (51%), bones (31%) and liver (22%). Metastatic GBMs are more frequent in young patients, maybe because they could have degenerated from a disseminated not treated low-grade glioma (15).

2.3. Histopathology

The microscopic histology of GBM is very heterogeneous and has clear signs of malignancy. There are multiple glial cells, little sizes, packed and pleomorphic. This shows up the rapid tumor growth (2). We can see multiple other signs of malignancy, such as: vascular occlusion, probably secondary to intratumoral vascular thrombosis, hemorrhage, hypoxia around vascular occlusion, creating a peripheric movement in palisade form, death of non-migrated cells creating areas of necrosis (pseudopalidaising necrosis (16)), secretion of proangiogenics, like vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), by hypoxic palisade cells, and an angiogenic response in peripheral regions and tumor growth to the neovascularized regions. VEGF has been seen to be more expressed in GBMP, but not only, and has been studied as a potential treatment target (8,11,12,16).

GBM is a highly vascularized angiogenic tumor that induces a variety of abnormalities that cause vascular dysregulation (10). During abluminal cell migration, glioma cells surround existing blood vessels and displace the astrocytic end-feet covering the vascular surface, which leads to destabilization of the basement membrane and down-regulation of endothelial tight junctions causing a breach of the blood-brain barrier (BBB) (17,18).

2.4. Clinical presentation

The symptoms of GBM can be very heterogeneous depending on his location. The most important manifestations are (19):

- Confusional state
- Memory loss
- Changes in personality
- Motor and sensitive deficit
- Intracranial hypertension signs: vomits, queasiness, papilledema and headache, which is present in 50% of patients at diagnostic, tends to be unilateral and has an increasing intensity. An abrupt intense headache in patients older than 50 years old should make suspect a malignant origin.
- Epileptic seizures: 50%, they mostly are partial seizures but they can generalize. They can be provoked by tumor's necrosis and hemosiderin leftovers from the hemorrhage. 77% can be controlled after the surgical resection. A bad control of episodes increases morbimortality of these patients (20).

2.5. Important classifications

Status of IDH

IDH1 and, sometimes, 2 are assessed in all GBM in clinical practise since the 2016 WHO actualization of brain tumors. GBM is nowadays classified:

- GBM, IDH-wild type (about 90% of cases) corresponding most frequently to the clinically defined primary or de novo GBM and predominant in patients aged over 55 years.
- GBM, IDH-mutant (about 10% of cases) corresponding closely to the so-called secondary GBM, with a history of prior lower grade diffuse glioma, and preferentially occurring in younger patients.
- GBM, NOS, a diagnosis that is reserved for those tumors for which full IDH evaluation cannot be performed (8). Author's note: almost all GBM figures form 3.2. (MRI as a diagnostic test for GBM) are described as GBM NOS as they were diagnosed before 2016).

Cell of origin

- Primary ("de novo")

Primary GBM (GBMP) affects elderly patients at a much higher occurrence and develops de novo without any history of similar diseases of lower grade, often presenting no symptoms before gaining malignancy.

Amounting to 90% of total GBM cases worldwide, primary GBM confers a worse prognosis compared to its secondary counterpart. This subset of GBM often shows molecular overexpression and amplification of epidermal growth factor receptor (EGFR), loss of heterozygosity (LOH) 10q, p16INK4A and phosphatase and tensin homolog (PTEN) mutations (8,21).

- Secondary

Secondary GBM (GBMS) makes up 5% of known cases globally and often affects younger patients, mean age of 45 y and is more frequent in men and in frontal lobe.

Genetic alterations more commonly seen in secondary GBM include IDH1 mutations, TP53 mutations, and 19q loss (8,21). The presence of a mutated IDH is associated to GBMSs and to a better prognostic (22). The time to progression from Anaplasyc astrocytoma (AA) a grade III to GBM is of 2 years, and the evolution of low grade astrocytoma (grade II) to GBM is of about 5 years (23).

They are histopathologicaly indistinguishable. One of the biggest differences between them is the mutation of IDH 1 and 2 (Figure 1). GBMPs are in 90-95% of cases not mutated (wild-type), as opposed, GBMSs present a muted IDH in the 70-80% of cases, Interestingly, IDH1 mutations are also found in 80% of diffuse astrocytoma and AA, the precursors of secondary GBM (8).

	Primary glioblastoma	Secondary glioblastoma
Expression profile	Classical, mesenchymal, neural	Proneural
Genomic alterations	TERT mutation	IDH1/2 mutation
		ATRX/TP53 mutation
	EGFR amplification	CIC/FUBP1 mutation
	NF1 loss	PDGFRA amplification
		1p/19q loss
Methylation status	G-CIMP negative	G-CIMP positive
Clinical outcome	Poor outcome	Improved outcome
Patient age	Older age	Younger age

TERT: Telomerase reverse transcriptase; ATRX: Alpha thalassemia/mental retardation syndrome X-linked; EGFR: Epidermal growth factor receptor; CIC: Capicua transcriptional repressor; FUBP1: Far upstream element binding protein 1; NF1: Neurofibromin 1; PDGFRA: Platelet-derived growth factor receptor alpha; G-CIMP: Glioma-CpG island hypermethylator phenotype; IDH: Isocitrate dehydrogenase

Figure 1. Principal differential characteristics of primary and secondary GBM from Li Q. (67)

2.6. Genetics

The genetic alterations and protein expression profiles in GBM were observed via various computational methods of large studies, leading to the establishment of a huge database of biomarkers of various classes (1,6,10,21). The principal genetic aberrations presented in GBM are described in Table 1.

- IDH1/IDH2 Mutations

IDHs (IDH-1 and IDH-2) catalyse the oxidative decarboxylation of isocitrate to a-ketoglutarate and reduce NADþ and NADPþ to NADH and NADPH (Figure 2), respectively.

Both IDH1 and IDH2 mutations are more frequently detected among grade II to III gliomas and GBMS (70% to 75%), whereas in GBMP represented only (5%). IDH1 mutations in diffuse gliomas are strongly associated with TP53 mutation and also codelection (1p)/(19q). Other genetic associations that have been reported in gliomas include IDH1 mutation and MGMT promoter methylation, in the absence of chromosome

10 losses and EGFR amplification. These associations might contribute to explain why IDH1/IDH2 mutations have been found to be positive prognostic factors (21,24).

The mutated IDH1 would cause eventual mutations in other genes, including the ATM gene and mTOR gene, the latter being a kinase closely associated with the GBM pathogenesis (9,18)

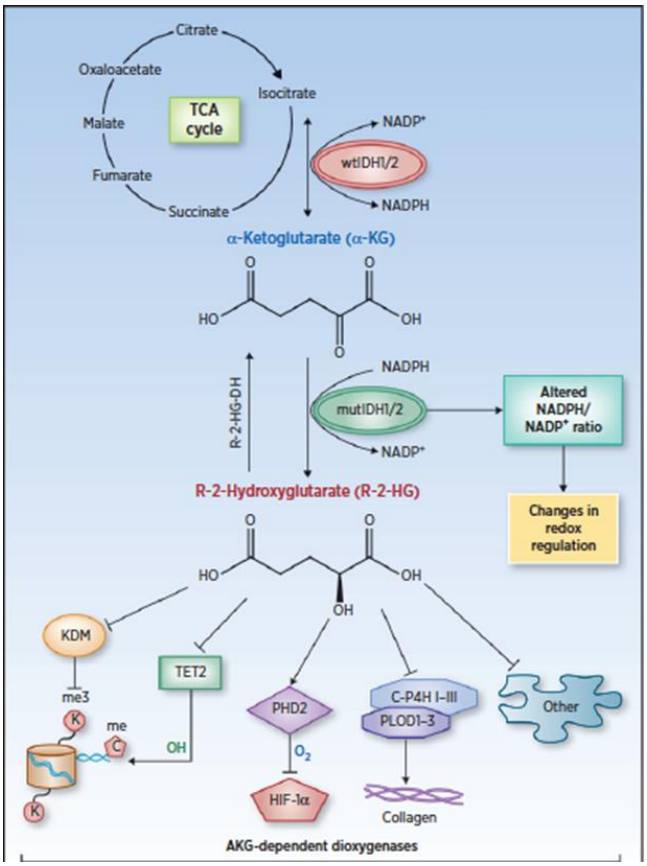


Figure 2. Molecular mechanisms of IDH associated tumorigenesis. From Owen, C. (68).

- Methylation of the MGMT Promoter

The MGMT gene is sometimes silenced in GBM by promoter hypermethylation in association or not with monosomy 10/del(10q). MGMT methylation was detected in 75% of GBMS, significantly more frequently than in GBMP (36%)(25). Currently, this represents one of the most relevant good prognostic factor in GBM and a potent predictor of response to treatment with alkylating agents such as Temozolomide (TMZ) a nitrosourea compound (21,24).

- Epidermal growth factor receptor (EGFR)

The amplification of EGFR and the genetic rearrangement of EGFR (EGFRvIII) are common hallmarks of GBM (40–50%), especially in GBMP (25). EGFR can activate pathways that are essential for GBM tumor cells to flourish, such as the receptor tyrosine kinase/Ras/phosphoinositide 3-kinase (RTK/RAS/PI3K) pathway (24).

- Tumor protein 53 (TP53)

TP53 gene encodes for a widely known tumor suppressor protein, p53. TP53 point mutations have been observed at a much higher rate in GBMS (90%) when compared to cases in GBMP (30). Mutations leading up to GBM might occur early in the development of gliomas and accumulate as the tumor progresses (23).

- Alpha-thalassemia/mental retardation syndrome X-linked (ATRX)

ATRX is a DNA helicase that plays a role in chromatin modulation and maintenance of telomeres. In adults, ATRX mutations have been seen in 75% of secondary GBM. ATRX mutations are mutually exclusive from 1p/19q codeletion but associated with p53 mutation, suggesting ATRX may drive lineage specific formation of astrocytoma (26).

- Loss of heterozygosity (LOH) of chromosome 10

This LOH 10q is presented in 70% of total GBM cases, although mostly in GBMP. It affects the tumor suppressor genes and subsequently leading to decreased protection of bodily systems toward tumorigenesis. LOH 1p and LOH 19q are molecular predictors of oligodendrocyte neoplasms for that reason no provide prognostic or predictive significance in GBM biomarkers (23).

Table 1. Overview of some of the most characteristic gene alterations in GBM, among others. Adapted from Park, S, Haque, A. and Deng, L. (25,27,28)

Genes aberrations	IDH- wildtype GBM (%)	IDH- mutant GBM (%)	Overall % in GBM
IDH1 mutation	5-6	> 95	7
IDH2 mutation	-	2-6	<0,1
TP53 mutation	25-35	62-65	50–60
ATRX mutation	3	90-95	70
EGFR amp	35-45	4	40–60
MGMT methylation	48.5	60-80	50–60

3. RADIOGENOMICS

Radiogenomics is a field of science that investigates the links between radiological features and the genomic profile of a tumor as a non-invasive replacement and/or complementary of histological confirmation (29).

Imaging features, such as mass effect, brain tumor contrast enhancement, degree of T2 edema, and contrast to T2 ratio, extracted from standard and advanced preoperative MR sequences via advanced computational methods have shown evidence to predict survival, molecular subtype, and mutational status in GBM (12,30,31).

IDH1 mutations in astrocytic neoplasms were also found to be associated with radiological characteristics including contrast enhancement, cysts, satellite lesions, frontal-lobe location, sharp tumor margins, and homogeneous signal intensity (10).

Heterogeneity

GBMs have significant genetic intratumoral and intertumoral heterogeneity (8). This heterogeneity, which is thought to arise from clonal expansion of multiple genetically divergent tumor populations, has implications for targeted therapy and tumoral resistance, and therefore in prognosis (31).

GBM contains a subpopulation of highly tumorigenic cells (GBM stem cells) from which recurrent GBM is thought to derive, and that give GBM the capacity to differentiate into multiple lineages of tumor genesis so called “many tumors in one (8,12).

3.1. MRI biomarkers

The use of MRI has recently expanded to create non-invasive imaging biomarkers of cellular/molecular characteristics, since imaging phenotypes can be correlated with genomic signatures, and such phenotypes can serve as non-invasive biomarkers of cellular gene expression. Thus, MRI can be used to link molecular and imaging diagnostics as a means to further refine and potentially provide for a non-invasive method of diagnosis and prognostication, and for elucidating specific therapeutic targets (32). Tissue blood volume, diffusion indices, and metabolic biomarkers yield specific information about tumor vascularity, cellularity, metabolic tumor heterogeneity, and other histologic properties (33).

Some of the most established and promising MRI biomarkers are:

- Relative cerebral blood volume (rCBV): rCBV metric is an accurate predictor of tumor grade (33).

- Normalized apparent diffusion coefficient (nADC).
- Spectroscopy metrics choline/creatine (Cho/Cr) and choline/N-acetyl aspartate (Cho/NAA): for high- and low-grade gliomas (HGG, LGG) and metastases differentiation (33).
- Peritumoral edema: could be the most useful for HGG vs.metastases differentiation (33).
- Tumor-associated neovascularization: detected on high-resolution blood-pool-contrast-enhanced magnetic resonance angiography (MRA) is useful biomarker that correlates with worse survival (17).

Future Biomarkers

- **Lipid droplets**

Lipid droplets (LD) are subcellular organelles that are the major storage site of neutral lipids, triglycerides and cholesteryl esters. Recent progress has revealed that lipid metabolism is rewired in GBM and promotes tumor growth. Magnetic resonance spectroscopy could be a possible tool to prove LD presence in a tumoral lesion (34).

3.2. Imaging in GBM diagnosis

Most patients with GBM undergo computed tomography (CT) of the brain upon initial presentation.

Signs of GBM in CT, typical presentation of GBM includes (Figure 3) (11):

- Irregular thick margins: iso- to slightly hyperattenuating (high cellularity)
- Irregular hypodense center representing necrosis
- Marked mass effect
- Surrounding vasogenic edema
- Hemorrhage is occasionally seen
- Intense irregular, heterogeneous enhancement of the margins is almost always present

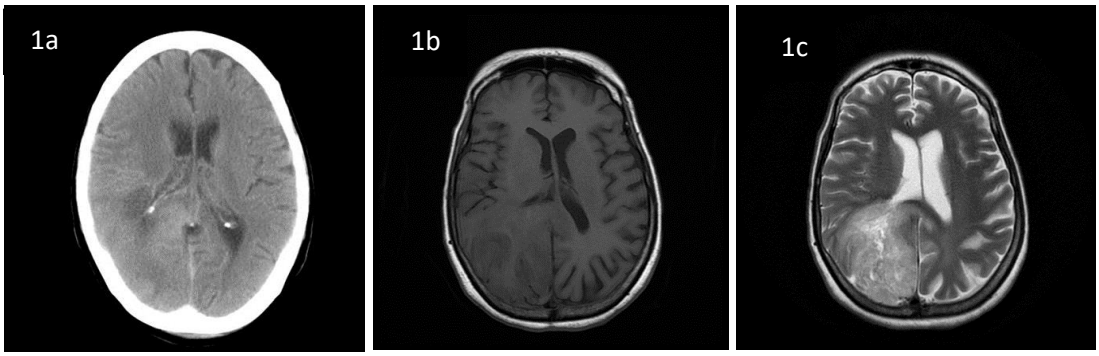


Figure 3. **1**: Glioblastoma NOS; 65 years; Female. Presentation: Left sided weakness, headache and unsteady gait. **1a** axial TC; **1b** axial T1 WI; **1c** axial T2 WI. Adapted from: case of Dr Mohammad A. ElBeialy, Radiopaedia.org, rID: 23473

Once a mass is identified and hemorrhage is excluded, a contrast-enhanced MRI is typically ordered, with standard T2-weighted (T2WI), T2-fluid-attenuated inversion recovery (T2-FLAIR), gradient echo (T2 GRE), T1-weighted (T1WI), and T2WI gradient echo (GRE), diffusion weighted sequences (DWI), perfusion weighted imaging (PWI) – dynamic contrast susceptibility (DCS) – relative cerebral blood volume (rCBV) and T1-weighted contrast-enhanced (T1CE) sequences (12).

Although sufficient sample from a biopsy is enough for GBM diagnosis confirmation, MRI has become a standard tool for brain imaging because it provides high resolution images with good contrast between different tissues (35).

MRI is the best imaging tool to characterize GBM and cerebral tumors and their differential diagnosis in general as is the most sensitive diagnostic test. It can be used as the diagnostic test, as a presurgical tool to guide biopsy and increase its accuracy, and to make a neuronal functional map for a minimum aggressive resection.

GBM is described in general as a heterogeneous, irregular big mass with contrast enhancement areas, peritumoral edema and a central area of necrosis. It is normally located in the supratentorial level, in one hemisphere, from there it can extend to the opposite one, through the corpus callosum, or to the brain stem (12). It is frequently a unique lesion, but in nearly 20% of cases it can be multifocal, seen as multiple contrast enhancement areas connected by white matter areas with altered signal, that corresponds to the microscopic dissemination of the tumor (36,37). It also exists the multicentric GBM in which contrast-enhancement areas are not connected (58,59).

Each MRI pulse sequence play an important role paper in the radiomic characterization of GBM, allowing to assess it through different perspectives:

- T1WI

  Is an anatomic pulse sequence. GBM shows as an irregular isointense, hypointense white matter mass (Figure 4). Necrosis, cysts and thick irregular margin. May have subacute hemorrhage (38,39).

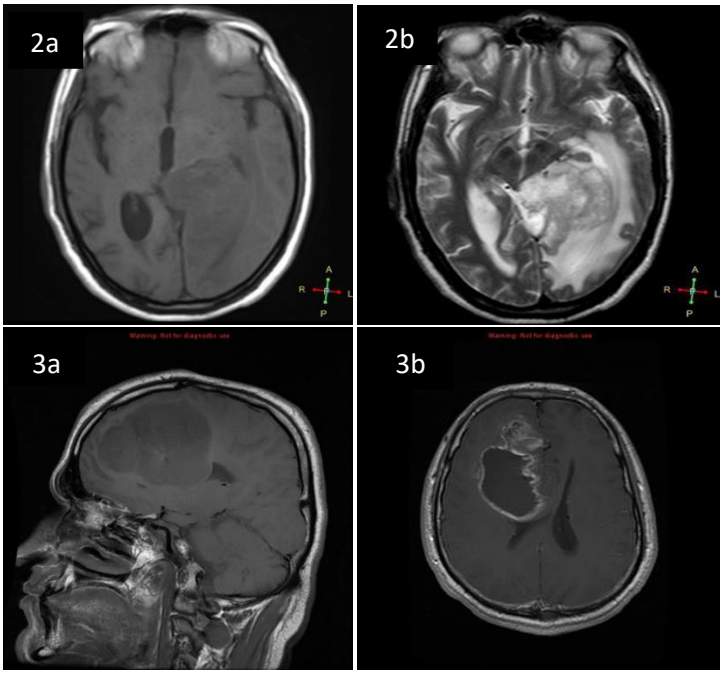


Figure 4. **2**: Glioblastoma NOS; 65-year-old, male. Presentation: headache, disturbed consciousness and memory. **2a:** axial T1 WI; **2b:** axial T2. **3**: Glioblastoma NOS; 25-year-old, male. Presentation: headaches and seizure. **3a**: sagittal T1 WI; **3b:** axial T1 CE. Adapted from: Case of Dr Ahmed Abdrabou, Radiopaedia.org, rID: 22779

- T1CE

T1 CE is based in images obtained after the administration of contrast (gadolinium) in an arterial phase. In patients with brain tumors, contrast-enhanced MRI with gadolinium-based agents are essential for planning surgical resection, delineating radiosurgical target volumes, and following patients for disease recurrence, as well as to provide a detailed description of possible hemorrhage, necrosis and the mass effect of individual malignancies that may be related to tumor aggressiveness. Moreover, information from contrast-enhanced MRI can be used to inform therapeutic decisions and monitor treatment outcomes (10,30,40).

Enhancement of intra-axial brain tumors is primarily based on disruption of the BBB and/or abnormal neovascularity, which allows the agent to accumulate within a lesion (Figure 5). The infiltrative nature of glioblastomas makes it difficult to delineate their margins and extension (8). Vascular regulation is impaired in contrast-enhancing (BBB breached) regions and appears normal in non-enhancing (BBB intact) regions (11,41).

GBM presents a thick, irregular rind of enhancement surrounding central necrosis typical. Enhancement may be solid, ring, nodular or patchy (11,39).

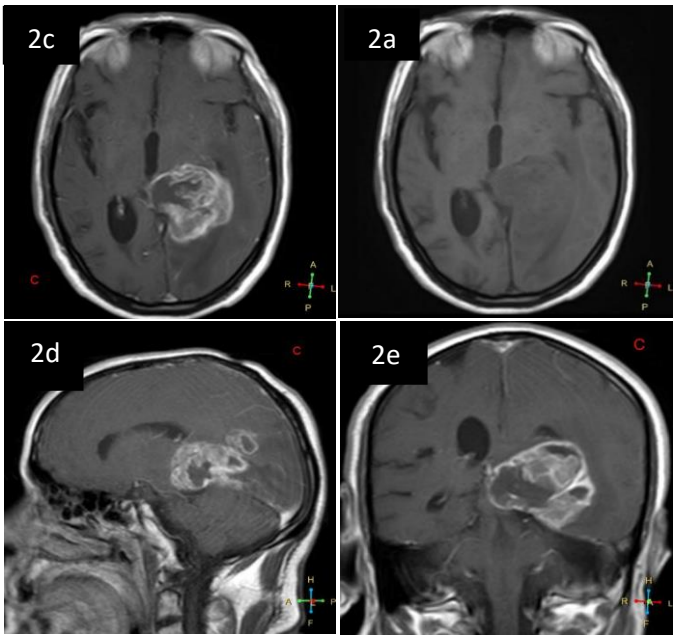


Figure 5. **2: 2c**: axial T1 CEWI; **2d**: sagittal T1 CE; **2e:** coronal T1 CE. Adapted from: Case courtesy of Dr Ahmed Abdrabou, Radiopaedia.org, rID: 22779

- T2 WI

It is also an anatomical pulse sequence, but white matter is seen darker than grey matter compared with T1WI (41). GBM is seen as a heterogeneous, hyperintense mass with adjacent tumor infiltration/vasogenic edema (Figure 6). Viable tumor extends far beyond signal changes. Necrosis, cysts, hemorrhage, fluid/debris levels, flow voids (neovascularity) may be seen (11,39).

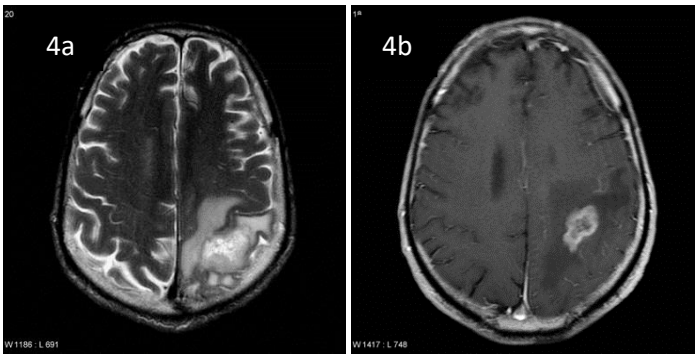


Figure 6. 4: Gliosarcoma, adult. **4a:** axial T2 WI; **4b**: axial T1 CE. Adapted from: Case of A.Prof Frank Gaillard, Radiopaedia.org, rID: 5322

- T2 FLAIR

T2 FLAIR supresses the cerebrospinal fluid signal and is used to demarcate peritumoral edema in the BAT (31). Peritumoral edema, exhibits few abnormalities in cerebral blood flow, cerebral blood volume, or permeability (18). Beyond the tumor bulk, pathology studies demonstrate microscopic tumor infiltration throughout the peritumoral edema, because edema appears to develop in response to angiogenic and vascular permeability factors associated with infiltrating tumor. More than 90% of tumor recurrences will occur within this T2-FLAIR envelope (12).

GBM presents heterogeneous, hyperintense mass with adjacent tumor infiltration/vasogenic edema (Figure 7). Comparing cerebral autopsy samples with the images of T2 FLAIR has been seen that the areas of peritumoral edema are areas of tumoral infiltration.

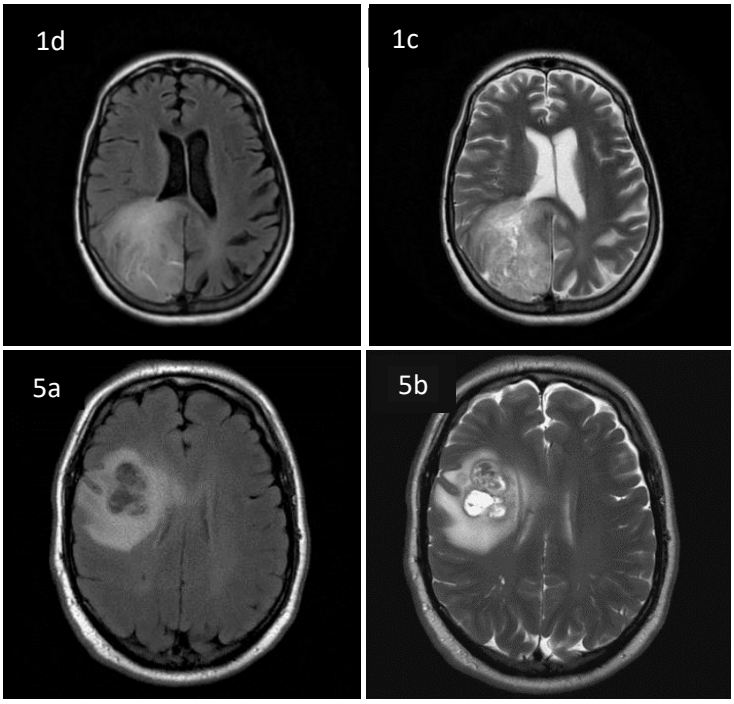


Figure 7. 1: 1d: axial T2 FLAIR; **1c:** T2 WI. **5:** Glioblastoma NOS, 55-year-old, male. Presentation: headaches. **5a**: axial T2 FLAIR; **5b:** T2 WI. Adapted from: 1: case of Dr Mohammad A. ElBeialy, Radiopaedia.org, rID: 23473 and **5:** Case of A.Prof Frank Gaillard, Radiopaedia.org, rID: 5322

- T2 GRE

Gradient echo sequences (GRE) are an alternative technique to spin echo sequences (T1 WI, T2 WI). Magnetic susceptibility artefacts are more pronounced on GRE sequences that on spin echo sequences (11,42). In some cases, GBM presents an artefact on T2 GRE related to blood products (Figure 8).

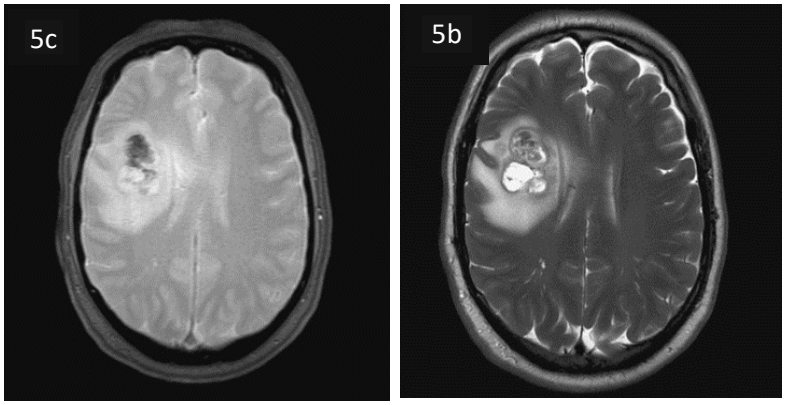


Figure 8. 5: 5c: axial T2 GRE; **5b**: axial T2 FLAIR. Adapted from: Case of A.Prof Frank Gaillard, Radiopaedia.org, rID: 5322

- DWI

DWI sequences are based on the measure of water diffusion in the cerebral tissue. The apparent diffusion coefficient (ADC) is a reconstruction of DWI that is related to the tissue cellularity. Hyperdense areas in ADC are regions of low cellularity and hypodense areas are regions of high cellularity (Figure 9). DWI is useful to identify areas of high cellularity and areas of necrosis (17).

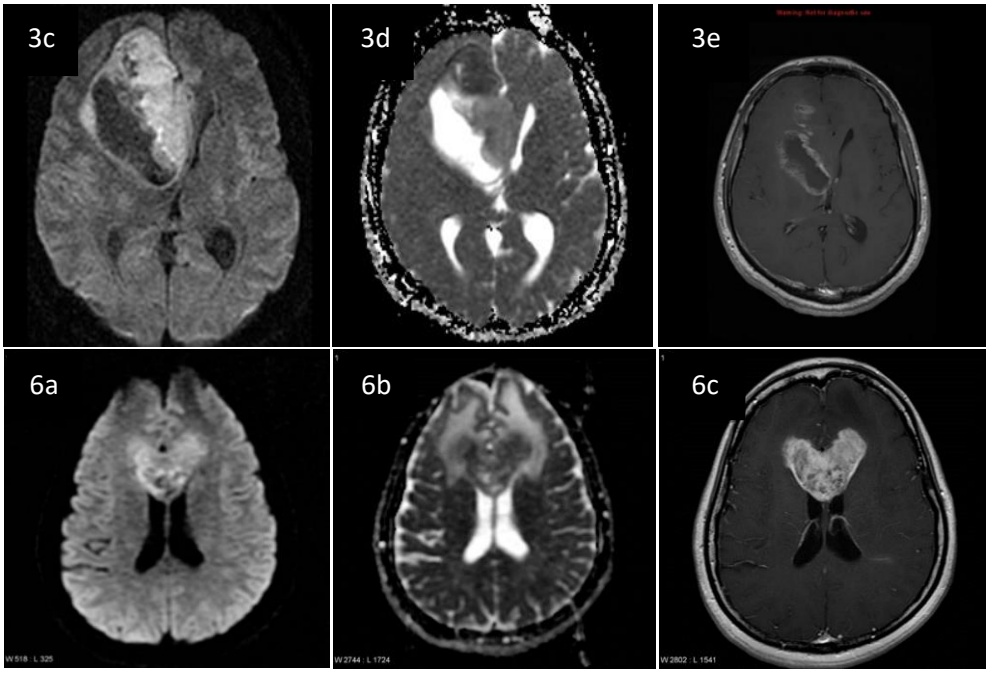


Figure 9. 3: 3c: axial DWI; **3d:** axial ADC; **3e**: axial T1 CEWI. **6**: GBM NOS with subarachnoid spread, with giant cell GBM component, adult. **6a**: axial DWI; **6b**: axial ADC; **6c**: axial T1 CE. Adapted from: **3:** Case of Dr Ahmed Abdrabou, Radiopaedia.org, rID: 22779 and from: **6:** Case of A.Prof Frank Gaillard, Radiopaedia.org, rID: 5290

ADC values may also be associated with poor response to treatment and worse survival among high grade glioma patients (12). A decrease in ADC in controls is related to a tumoral progression (43,44). GBM has lower measured ADC than LGG, and no predominant diffusion restriction typically.

- PWI

PWI is typically used in conjunction with conventional imaging to provide additional information about cerebral hemodynamics (9). The most used technique is DCS that enables to calculate the relative cerebral blood flow (rCBF) and CBV in a region of interest (42–44). Alterations in CBF and CBV due to the increased vascularization of tumors may be biomarkers for intracranial lesions and are related to microvessel morphology (31,48).

Several imaging methods have been proposed to evaluate the overall degree of tumor-associated neovascularization either directly by digital subtraction angiography (DSA) or magnetic resonance angiography (MRA) or indirectly by DSC perfusion MRI measuring CBV or CBF in tumor areas (9). CBV is difficult to calculate that is why in most instances what is actually being calculated is CBV relative (rCBV) to an internal control, like contralateral normal white matter for example (47).

GBM has an elevated maximum rCBV (Figure 10) compared to low grade glioma and increased permeability compared to low grade gliomas, which may help asses tumor grade.

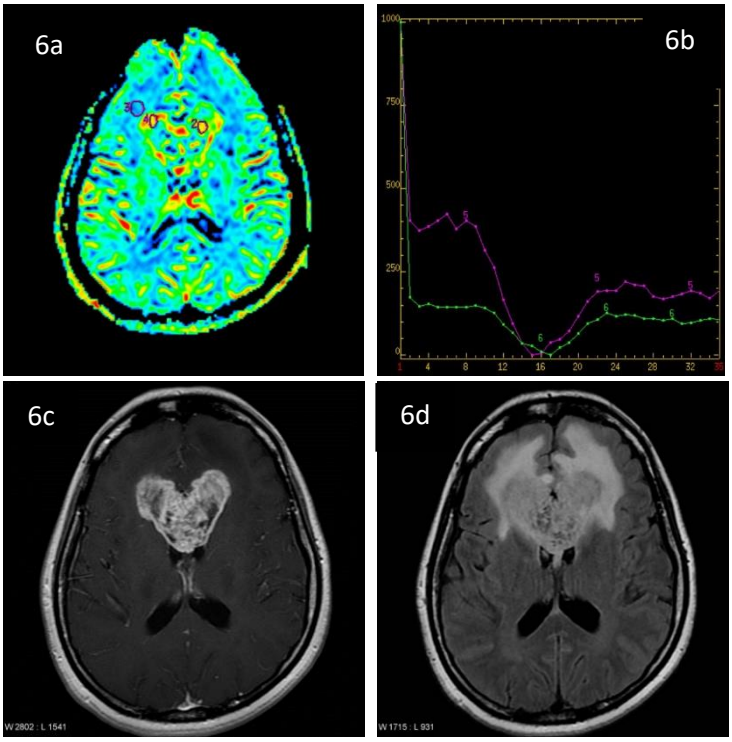


Figure 10. **6: 6a:** axial CBV ROI 2-4; **6b:** CBV graphic ROI 2-4; **6c:** axial T1 CEWI; **6d:** axial T2 FLAIR. Adapted from: Case of A.Prof Frank Gaillard, Radiopaedia.org, rID: 5290

- MRA

CE MRA is a technique involving 3D spoiled gradient-echo (GE) sequences, with administration of gadolinium-based contrast agents (GBCA). It can be used to assess vascular structures of almost any part of the body (49).

There is a significant relationship between maximum rCBV ratio and angiographic vascularity on MRA in gliomas (17). MRA and other non-invasive imaging techniques such as DSC perfusion MRI can provide a more global view of tumor neovascularization in vivo (17). In figure 11 a normal brain MRA highlights the anterior cerebral circulation.

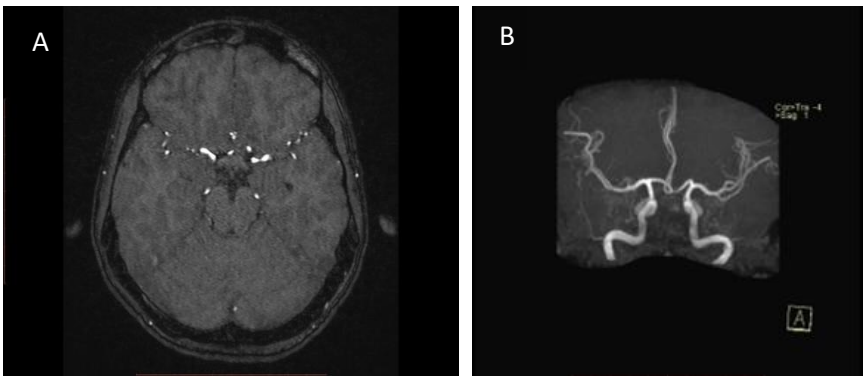


Figure 11. MRA of a normal brain. **A**: axial MRA; **B**: reconstructed anterior circulation MRA Adapted from: Case of Dr. Hidayatullah Hamidi, Radiopaedia.org, rID: 53690

3.3. Radiological pseudoprogression

Pseudoprogression is a radiological concept where the tumor's contrast enhancement area increases compared to the last posttreatment control MRI, followed by a stabilization or reduction without any treatment. Nearly 30% of GBM treated with radiotherapy (RT) and temozolomide (TMZ) present it. It is caused by the temporal interruption in myelin synthesis secondary to the RT effects over oligodendrocytes. The exact mechanisms are still unknown.

It appears more frequently in the first 3 months after concomitant radiotherapy (RT) and chemotherapy (QT) treatment, but it has been seen even past 6 months. It is most commonly observed in patients whose tumors harboured a methylated MGMT (up to 90%)(12). Some studies support that DCS sequences may be useful to differentiate progression of pseduoprogression in up to 90% of cases, furthermore they could differentiate radionecrosis from tumoral recurrence (50–55).

3.4. Radionecrosis

Radionecrosis is a severe local reaction to RT that shows up as alteration of CBB, edema, mass effect, seeming a disease progression. Radionecrosis is located in white matter, it is associated with calcifications, fibrin deposit, vascular hyalinization and vascular thinning. The final phase is a chronic inflammatory process, with oxidative stress, that inhibits neurogenesis.

Its incidence is about 6% of all GBM that receive RT. It can appear from 3 months to 3 tears after RT and can be related to: high doses of radiation (>50 Gy), high fractionated volumes (>2,5 Gy/day) and concomitant treatment with QT. It shows a low rCBV area in MRI.

Pseudoprogresion and radionecrosis are processes related to antitumoral response to RT/QT and to patients recover. The differentiation between real tumoral progression and pseudopreogression/radionecrosis still difficult (52).

4. TREATMENT

The standard treatment of GBM consists of (see Figure 13, Annex 3):

- Maximum surgical resection followed by fractioned local RT, 2 Gy/day during 6 weeks (total dose of 60 Gy). With concomitant daily TMZ (75mg/m2/day) from the first day of RT to the last, as radiosensitizer, not surpassing 49 days. After 4 rest weeks, 6 adjuvant cycles of TMZ (200 mg/m2/day) for 5 days /28 days (Stupp scheme) (56).
- It remains impossible to cure GBM, which led patients to opt for other treatment options to alleviate GBM symptoms, such as dexamethasone (21).

Surgery

Gross total resection (GTR) is defined as the total resection of the contrast-enhanced area in T1 CE MRI. It can be accomplished in up to 40% of GBM (57).

Surgical resection in GBM is a big prognostic factor, but is difficult due to his tendency to infiltrate cerebral parenchyma. Tumor cells can be found outside the contrast-enhancing region with density decreasing with distance and extent of resection correlates with clinical outcome (18).

The main contraindications to resective surgery are poor performance status (Karnofsky<60) and eloquent location (8,9).

Radiotherapy

Radiotherapy planning includes registration (also known as “fusion”) of the post-operative MRI (T1CE and T2-FLAIR sequences) with the planning simulation CT, which allows for delineation of the T2-FLAIR abnormality and residual enhancement in treatment planning (12). RT has to start 4/5 weeks after surgery because cerebral tissue need to reoxygenate after a surgical aggression as it has been seen that tumoral tissue can be radioresistant because of the edema and the secondary hypoxia of surgery (58).

Chemotherapy

- Temozolomide (TMZ): is an alkylating agent methylator of DNA, inducing cell apoptosis. It is used as first line in the treatment of GBM (59).

  MGMT promoter methylation is associated with low levels of MGMT proteins, and, consequently, reduces DNA repair activity against alkylating agents. Therefore, glioblastomas with MGMT promoter methylation can be expected to have a more favorable response to a DNA alkylating. In fact, MGMT promoter methylation is a predic-

tive factor of TMZ effectiveness, patients whit MGMT promoter methylation have better response to TMZ with concomitant RT but not having MGMT promoter methylated does not change treatment (60,61).

- Antiangiogenics: VEGF inhibitors (bevacizumab) can achieve a normalization in tumoral vessels permeability, which gets an anti-inflammatory effect. This reduction in permeability may cause an error interpreting MRI images, what it is called as, pseudoresponse, a decrease in contrast enhancement area of the tumor making think is caused by a positive response to treatment (62,63). Bevacizumab with irinotecan is the treatment after progression to TMZ.

Treatment response evaluation

MRI continues being the best option to follow-up and asses treatment response and recurrence with a radiologic control done every 12 weeks (52). The moment of tumor progression is marked by the time when it was first suspected. The clinical state follow-up is performed with KPS (see Figure 14, Annex 4), Mini-Mental State test (MMS) (see Figure 15, Annex 4) and Barthel test (see Figure 16, Annex 4) (68).

To distinguish between radionecrosis/pseudoprogresion/real tumoral progression MRI gives a lot of information but clinical information is still a fundamental tool to differentiate them.

4.1. Treatment of recurrence

Surgery: is only recommended in 20-30% of the patients (64).

Radiotherapy: is controversial. Only patients with clinical and imaging characteristics of bad prognostic are given palliative doses of RT (30-36 Gy, not surpassing 100 Gy) (65).

Chemotherapy: nitrosoureas, TMZ or antiangiogenics (bevacizumab +/- irinotecan or lomustine can be used. Studies that only take into account the contrast-enhanced region as a prognostic marker did not fins associations, because bevacizumab affects the peritumoral edema (T2 FLAIR) (51). TMZ rechallenge could be a valid treatment option for patients with recurrent GBM and the combination of high CBF and MGMT promoter methylation, but not with the combination of low CBF and no MGMT promoter methylation (61).

5. JUSTIFICATION

GBM is the most frequent malignant primary brain tumor and the deadliest with a mean overall survival of 14 months and less than 5% surviving more than 5 years following diagnostic. His location and microscopical infiltration make the surgical resection almost impossible and the intratumoral heterogeneity makes the recurrence inevitable.

Although there are described relations between radiomics and molecular and genetic profiles of GBM and between radiomics and patient's prognosis, to date there is no way to identify molecular profile of GBM by MRI.

Peritumoral edema size and necrosis have been both related with survival in several studies, as well as total tumor volume, age, contrast-enhancement regions and molecular status, among other. But no explanation for the big difference in patient's survival of GBM has been demonstrated yet.

It is interesting to analyse all of these characteristics, that define GBM from different fields, together in a multicentre, prospective cohort study with a sample of 200 patients.

No studies have studied the relations between radiomics, genetic profiles and prognosis of GBM in Catalan population, and few studies have clustered MRI patterns and compared the IDH1 status and MGMT promoter methylation together in a prospective study with 3 years of follow-up.

Thus, this protocol will try to correlate MRI patterns (necrosis, contrast-enhancement, rCBV, peritumoral edema and tumor neovascularization) with molecular features (IDH status and MGMT methylation) to find associations that permit their prediction by MRI, as well as with prognosis (OS and PFS). This study gives a new opportunity to find a way of predicting genetic profile of GBM through MRI patterns to simplify their diagnostic, in some cases avoid biopsy and its complications and start as briefly as possible the treatment, in addition to knowing its prognosis and giving real expectations to the patient.

6. HYPOTHESIS

Primary hypothesis

MRI patterns can predict molecular features (IDH status and MGMT promoter methylation) of GBM, in some cases avoiding the need for biopsy and therefore speeding up the certain diagnosis of GBM and improving patient's prognostic.

Secondary hypothesis

- MRI patters of GBM can be independently related to the patient's prognosis.

7. OBJECTIVES

Main objective

- To validate MRI patterns of GBM that predict molecular features IDH status and MGMT promoter methylation.

Secondary objectives

- To assess the correlation between MRI patterns of GBM and patient's prognosis.

8. SUBJECTS AND METHODS

8.1. Study design

This study will be a prospective, longitudinal, observational, multicentre, cohort during 6 years, analysing the relation between GBMs MRI images and its genomic profile and prognostic.

Three hospitals from ICO (Duran I Renyals hospital – ICO L'Hospitalet, Germans Trias I Pujol hospital – ICO Badalona, and Dr. Josep Trueta hospital – ICO Girona) will participate in this Project with the neuroradiology team of HJT de Girona as coordination centre.

8.2. Study population

All patients older than 18 years old diagnosed of GBM in any of the hospitals participating in the study will be asked to participate.

8.3. Subjects selection

Inclusion criteria

- Age > 18
- No prove of previous brain tumors
- No previous treatment
- No previous CNS diseases (epilepsy, cerebral stroke, cerebral metastasis)

Exclusion criteria

- Patients that can't follow the projects plan
- Allergic to gadolinium (rare)
- Diseases that contraindicate SNC surgery

8.4. Sampling

Sample size

The sampling size for this study are all the patients diagnosed of GBM in the 3 different hospitals of ICO that take part in this study. An intentional non-aleatory sampling will be done, informing every patient newly diagnosed of GBM about the study.

ICO is the reference centre for approximately the 45% of adult population in Catalunya (2.889.000). The expected number of patients in 2 years, taking into account that the incidence of GBM in Girona's (Catalonia) population is 4,17 cases per 100.000 inhabitants, are more or less 240 cases.

In a bilateral contrast with alfa=5% and assuming a moderate size effect with a sample of 240 cases the statistical power is 98%

Computations were carried out by Prof. Dr. Marc Saez's software based on the library pwr of the free statistical environment R (version 3.6.2).

Sample collection

All the patients diagnosed of GBM will be invited to participate in this project at the moment of diagnosis. One of the investigators, the responsible for each ICO hospital, will explain all the phases of the study and how is going to be done the follow-up. An informed consent will be given to sign (see Informed consent sheet in annex 6).

One investigator will determine, attending at the inclusion criteria, which patients will be included in the final sample to take part in the study.

8.5. Variables

The main variables analysed in the main objective are:

Dependent variables

- **IDH1 mutation status**: described as mutated/wild type, using immunohistochemistry.
- **MGMT promoter methylation**: described as methylated/not methylated, using PCR technique.

Independent variables

- **Necrosis**: described as yes or no. Defined as the presence of areas of high ADC values.
- **Contrast enhancement**: described as yes/no. Defined as the appearance of enhancement of the tumor in T1 CE.
- **Peritumoral edema ratio**: defined as the proportion between the maximum hyperintensity area in T2 FLAIR and the total tumor volume.
- **Necrosis volume**: in ml. Defined as areas with higher ADC values.
- **Tumoral perfusion (rCBV) in the necrosis area**: no units. Calculated with DSC placing regions of interest over necrosis areas and other over an internal control.
- **Perfusion (rCBV) in the peritumoral edema**: no units. Calculated with DSC placing a region of interest over peritumoral edema and other over an internal control.
- **Tumor-associated neovascularization**: defined as hypervascular, in tumors with more than three vessels or hypovascular in MRA.

The variables analysed in the secondary objectives are:

Dependent variables

- **OS:** in days. Defined as the days passed from the diagnosis till the patient's death.
- **PFS:** in days. Defined as the days passed from treatment till tumor progression appearance. The moment of progression is marked by the time when it was first suspected.
- **Radiological pseudoprogression**: described as yes/no/uncertain. Defined as an increase of tumor contrast enhancement area posttreatment (1-3 months) that reverts without any treatment.
- **Radionecrosis**: described as yes/no/uncertain. Defined as an increase of the contrast enhancement area and peritumoral edema in the white matter (3-12 months posttreatment) with a chronic inflammatory process.

Independent variables

- **Necrosis**: described as yes or no. Defined as the presence of areas of high ADC values.
- **Contrast enhancement**: described as yes/no. Defined as the appearance of enhancement of the tumor in T1 CE.
- **Peritumoral edema ratio**: defined as the proportion between the maximum hyperintensity area in T2 FLAIR and the total tumor volume.
- **Necrosis volume**: in ml. Defined as areas with higher ADC values.
- **Tumoral perfusion (rCBV) in the necrosis area**: no units. Calculated with DSC placing regions of interest over necrosis areas and other over an internal control.
- **Perfusion (rCBV) in the peritumoral edema**: no units. Calculated with DSC placing a region of interest over peritumoral edema and other over an internal control.
- **Tumor-associated neovascularization**: defined as hypervascular, in tumors with more than three vessels or hypovascular in MRA.

Co-variables

Main objective co-variables

- Sociodemographic variables:
  - **Age** → <70/>70 years
  - **Sex** → Male/female
- Clinical variables:
  - **KPS** at diagnosis and in follow-ups (Figure 14, Annex 4).
  - **MMS** at diagnosis and in follow-ups
  - **Barthel** test at diagnosis and in follow-ups
  - **Symptoms**: described as yes/no in the following ones: headache, vomits, neurologic focality and epileptic seizures.
  - **Dose of Corticosteroids received**

- Tumor related variables:
    - **Total tumor volume** → expressed in ml. Result of the sum of enhancing region area in T1CE and area of peritumoral edema in T2 FLAIR.
    - **Type of enhancement:** patterns of enhancement will be identified based on the morphology feature of the largest enhanced tumoral area on T1 CE.
        - Non-ring like: any other patter a part from ring like enhancement
        - Ring like: will be defined as cystic necrosis with peripheral enhancement.
        - Multifocal: more than one area of enhancement located separately from each of the other enhanced areas on a postcontrast T1 WI.
    - **Location** → described as supratentorial/Infratentorial based on the findings in anatomical MRI sequences (T1 WI, T2 WI).

<u>Secondary objectives co-variables</u>

- All **main objective co-variables**.
- **Type of treatment received**:
    - Surgery
    - RT: dose and scheme
    - QT: type and dose
- **Grade of surgical resection**: described as GTR or rT. Defining GTR as no visible contrast enhancement on postoperative MR images within 48 hours after surgery for contrast-enhanced tumors or the disappearance of all abnormal hyperintense changes on preoperative MR images for tumors not demonstrating contrast enhancement. Resections that are not GTR were considered residual tumors (rT). Measured by the comparison between presurgical MRI and postsurgical MRI contrast enhancement area T1 CE.

8.6. Data collection

Imaging data will be obtained from the Picture Archiving and Communication System (PACS), where all imaging tests of the patient are load up, regardless from which hospital it comes from and is accessible from HJT computers.

Neuro-oncology service of each hospital will be asked to make sure they write down in SAP all the sociodemographic and clinical variables and co-variables of the study to fill the data collection form at the end of the follow-up without errors (see Data collection form, annex 7).

Neuroradiologists of each hospital will be asked to make sure the following MRI images of the patient are loaded in PACS:

- Diagnosis
- Presurgical
- Postsurgical (<48h after surgery)
- Follow-up: every 12 weeks

The following data will be collected from the patient's SAP and then compared with the data collection form to check possible errors:

- Identification of the patient: age and sex
- Clinical state: symptoms, KPS, MMS, Barthel and dose of corticosteroids.
- Clinical information of the patient in each medical evaluation of the follow-up.
- Type of treatment and information about the grade of resection
- Molecular results of surgical tissue samples of GBM including: IDH status and methylation of MGMT.
- Date of progression
- Events: death

A personal and a relative's personal number and the personal and relative's direction will be asked together with the informed consent (see Informed consent sheet, Annex 6) to prevent losses in the follow-up.

Image acquisition

The images will be obtained with a 1,5 T MRI in all three hospitals. The MRI machine protocol parameters will be the same in all hospitals and are the following:

T1WI, FLAIR, single-shot echo-planar DWI, and firstpass DSC perfusion-weighted images with gadobutrol (Gadovist®) at 0.1 mmol per kilogram of body weight. Parameters were: for spin-echo T1-weighted imaging: TR/TE, 600 ms/ 10 ms; flip angle, 90°; and matrix, 256×192; and for FLAIR: TR, 9000 ms; TE, 114 ms; inversion delay, 2200 ms; flip angle, 90°; and matrix, 256×192. Section thickness was 5 mm with a 1-mm interslice gap and field of view was 230× 180 mm and brain coverage were 120 mm.

DWI

DWI (TR, 3758 ms; TE, 99 ms [b=0 and 1000 s/mm2]; 20 sections; section thickness, 5 mm; intersection gap, 1 mm; field of view, 230×230 mm; matrix, 192×128; two signals acquired) was performed in the axial plane. Images were acquired in three orthogonal directions and combined into a trace image. ADC maps were calculated on a voxel-by voxel basis.

DSC-MRI

DSC-MRI was performed with multislice T2* single-shot EPI technique applied for perfusion studies, with acquisition of images before, during, and after rapid administration of a contrast bolus (16 7-mm sections without gap; matrix, 128×128;field of view, 230×230 mm; TR, 1800 ms; TE, 25 ms; flip angle, 90°). The TR of each multishot block was 17 ms, and the acquisition time for each dynamic volume was 1.8 s. TE was 17ms and the flip angle 7°. Each perfusion series consisted of 50 dynamic acquisitions with temporal resolution set to 1.8 s during the first pass of a standard dose (0.1 mmol/ kg bolus of gadobutrol injected at 5 mL/s with a power injector, followed by a 20-mL bolus of saline at the same rate).

High-resolution contrast-enhanced MRA

To ensure gadobutrol clearance, we waited 48 h after gadobutrol injection to acquire 3D high-resolution contrast-enhanced MRA images of the entire head 5 min after administering 0.03 mmol/kg body weight of gadofosveset. 3D highresolution contrast-enhanced MRA was performed using a multishot spoiled turbo fast field echo sequence with low-high profile order. Voxel size was set to 0.6×0.6×0.6 mm and volume coverage to 150 mm; the coronal acquisition plane was used. To ensure optimal tissue background suppression, a saturation prepulse was used (turbo fast field echo shot interval 627 ms) combined with 180° inversion pulse frequency selective for fat suppression (inversion

delay 70 ms). Other scan parameters were: TR, 8.8 ms; TE, 2.6 ms; flip angle, 35°; TFE factor, 60; field of view 230×180 mm. Scanning took 5 min and 6 s.

8.7. Organization and procedures

Imaging analysis: identification of imaging features

Tumor variables will be studied by two experienced neuroradiologists blinded to the patient's clinical information. Another blinded neuroradiologist will re-examine the images and will determine which should be used if the first two radiologists disagree.

In the first 2 years, when sampling is going to be made, neuroradiologist will analyse diagnosis MRI of all the patients. After that, they will start with the presurgical and postsurgical MRI (to evaluate the grade of surgical resection) from the first patient to join the study, till the last one, over 3 years (duration of the follow-up). And then, follow-up MRI informed as abnormal will be analysed searching radiological signs of pseudoprogresion/radionecrosis/real tumor progression. Only in the cases where differentiation of this entities is not possible using only MRI, clinical information of the patient at that moment will be checked to help the analysis.

They will analyse the **independent variables**: Necrosis, contrast enhancement, peritumoral edema ratio, necrosis volume, tumoral perfusion (rCBV) in the necrosis area, perfusion (rCBV) in the peritumoral edema and tumor-associated neovascularization.

And the **MRI related co-variables**: Total tumor volume, type of enhancement and location.

Anatomopathological protocol

For the confirmed diagnosis of GBM a biopsy will be done before the inclusion of the patient to the study, so IDH status and MGMT promoter methylation will be already known. However, surgical tissue samples will be analysed to confirm the results as biopsies may not represent the molecular profile of the hole tumor.

After surgery, tissue samples of the tumor will be reviewed by a single anathopatologist (with at least 2 years of neuropathology experience) from each hospital for IDH status and MGMT promoter methylation, using Immunohistoquimic (which is available in all hospitals of the study) and polymerase chain reaction (PCR) respectively, except for HJT. Tissue samples of HJT will be sent to Germans Trias I Pujol university hospital (ICO

Badalona), as is the reference collaborator hospital where methylation status of MGMT is performed.

Patients with no molecular results for IDH status or MGMT promoter methylation, due to either lack of biopsy tissue sample or to an impossible surgical resection/surgical intervention, will be described as Unknown.

Follow-up

The clinical information in each periodical visit and in diagnostic, given by the neurology service of each hospital, will be revaluated by another physician collaborator of the research team blinded to all other variables and co-variables, except the ones resulting of knowing personally the patient (age, sex and clinical co-variables).

Each patient will be citated every month for a clinical evaluation and every 12 months for an MRI study. A worseness in the patient's symptoms will be useful to perform an extra MRI to assess if there are radiological signs of pseudoprogression/radionecrosis/real tumor progression.

If a patient does not come to any of the planned medical consultation, his/her physician will be asked to call the patient to know the reason of the absence and try to persuade him/her to come. If it's impossible to contact with the patient, a call to the relatives will be done. If it is still impossible to contact with the patient a presential visit will be done.

The follow-up would end if the patients wants it (at any time) or at his/her death.

After the collection of all the data, a data base will be created to make the statistical analysis easier. And then associations between MRI analysis results, molecular profile and prognosis will be searched using statistics.

9. STATISTICAL ANALYSIS

- **Descriptive analysis**

According to the main objective I will summarize the relationship between both of dependent variables (IDH, MGMT) that are dichotomic qualitative variables, and the quantitative independent variables **(**peritumoral edema ratio*,* necrosis volume, tumoral perfusion (rCBV) in the necrosis area, perfusion (rCBV) in the peritumoral edema) using the mean, the standard deviation and the median and the interquartilic range (IQR), when the distribution of the independent variable is symmetrical and asymmetrical respectively. Dependent variables will be summarized with proportions in a cross tab stratifying by the qualitative independent variables (necrosis, contrast-enhancement and tumor-associated neovascularization).

Regarding the second objective, the relationship between the qualitative dichotomic dependent variables (pseudoprogression, radionecrosis) and the independent ones will be summarized as outlined above.

The PFS and OS, quantitative discrete variables, will summarized using the Kaplan-Meier survival curve estimator, stratifying by the independent variables. These will be categorized in quartiles. In all cases, the analyses will be repeated stratification by covariables, the quantitative covariables will be categorized in quartiles.

- **Bivariate inference**

The association between the dependent variables and the qualitative independent variables will be assessed by means of the chi-squared test.

The relationships between the dependent variables of the main objective and the quantitative independent variables will be assessed by means of T-student (difference of means) and the Mann-Whitney's U (difference of medians), depending on the symmetry or asymmetry of the variable, respectively. The same procedure will be used for the relationship between the qualitative dependent variables and the independent variable of the second objective. The difference of the survival curves in Kapplan-Meier will be assessed by the log-rank test.

In all cases, the analyses will be repeated stratifying by covariables, the quantitative covariables will be categorized in quartiles.

- **Multivariate analysis**

The relationships between the dependent variables of the main objective and the quantitative and qualitative variables of the secondary objective will be adjusted in logistic regressions, controlling for the corresponding co-variables for each of the objectives.

With respect the relationship with the other dependent variables of the secondary objective, the independent variables will be adjusted using a Cox regression, controlling for the co-variables of this objective.

10. WORK PLAN AND CHRONOGRAM

10.1. Research team personnel

The research team will be composed by the general coordinator of the study, one extra coordinator for each hospital, 5 neuroradiologists working at Hospital Dr. Josep Trueta (3 of them with 10 years of experience) and 1 statistical analyst.

A project manager will be hired to manage patient's information, control that the patient's related variables are collected correctly and in the same way in all hospitals, control that the budget us followed as much as possible, ensure the compliance with timelines and milestones, act as the main line of communication between hospital collaborating professionals, reference investigators and the general coordinator and will provide input to study design, budget and ensures progress reporting.

10.2. Study stages

This study has an estimated duration of 6 years, including 6 months of study design and coordination, 2 years of sampling and MRI images analysis, 3 years of follow-up for every patient and 6 months for the execution of the results, conclusions and publication dissemination.

- **Stage 0: Protocol design (3 months)**
    - **1st step**: bibliographic research by the study coordinator (principal researcher - PR), basic hypothesis idea and decision of making a study about it.
    - **2nd step**: proposal to ICO and to neurology, neuroradiology and anatomopathology services of the three ICOs hospitals by the PR.
    - **3rd step**: protocol elaboration: study protocol development. Delimitation of the objectives, more bibliographic research about actual state of GBM diagnostic and treatment, specifically radiogenomics and prognostic related to radiomic and molecular parameters.

      And determination of reference investigators and collaborating professionals for the hospitals that accept participating in the study.
    - **4th step**: ask for a research scholarship to acquire founds to begin the study (3.500 € for the first 3 years).

- **Stage 1: Ethical approval (1,5 months)**
    - **5th step:** presentation of the protocol to the HJT's CEIC and to other hospitals CEIC as well as to ICO.
- **Stage 2: Data collection (5 years)**
    - **6th step**: 2 years of sample collection and signature of the informed consent. Recollection of the personal ID (identification document) and personal data. Furthermore, compilation and analysis of all MRI diagnostic images by JT hospital investigators and compilation of molecular features of each patient.
    - **7th step**: periodic videoconferences between all research team members (each 6 months) in order to evaluate the organization and accuracy in data collection and assemble that all hospitals are following the same protocol correctly. In each radiology congress where coordinators of the hospitals and the general coordinator coincide, they will take the opportunity to meet.
    - **8th step**: Research scholarship prorogation: As research scholarships are given for a maximum of 3 years, exceptionally of 4, another study protocol will be made to ask for a prorogation at 3 years of the beginning of the study (collection of the sample), more or less at half of the study. The data collected until then will be reviewed and exposed as a justification for founds to complete the study.
    - **9th step**: 3 years of follow-up, where clinic and treatment information, apparition of radionecrosis, pseudoprogression and recurrence will be documented. As well as analyse postsurgical MRI comparing it with the presurgical ones to assess GTR/rT.
- **Stage 3: Statistical analysis (4 months)**
    - **10th step**: Integration and computerization of results creating a common database to make the statistical analysis easier. Each patient will be given a number, to assure confidentiality, which will be associated with all the information collected for each patient.
    - **11th step**: The qualified statistical analyst will analyse during 3 months obtaining the preliminary results of the study.

- **Stage 4: Interpretation and discussion of the results (1 month)**

    - **12th step**: The principal investigators (general coordinator, neuroradiologists of HJTs hospital, and reference investigators and collaborators of each hospital) will have a general meeting to discuss and take conclusions about the results.

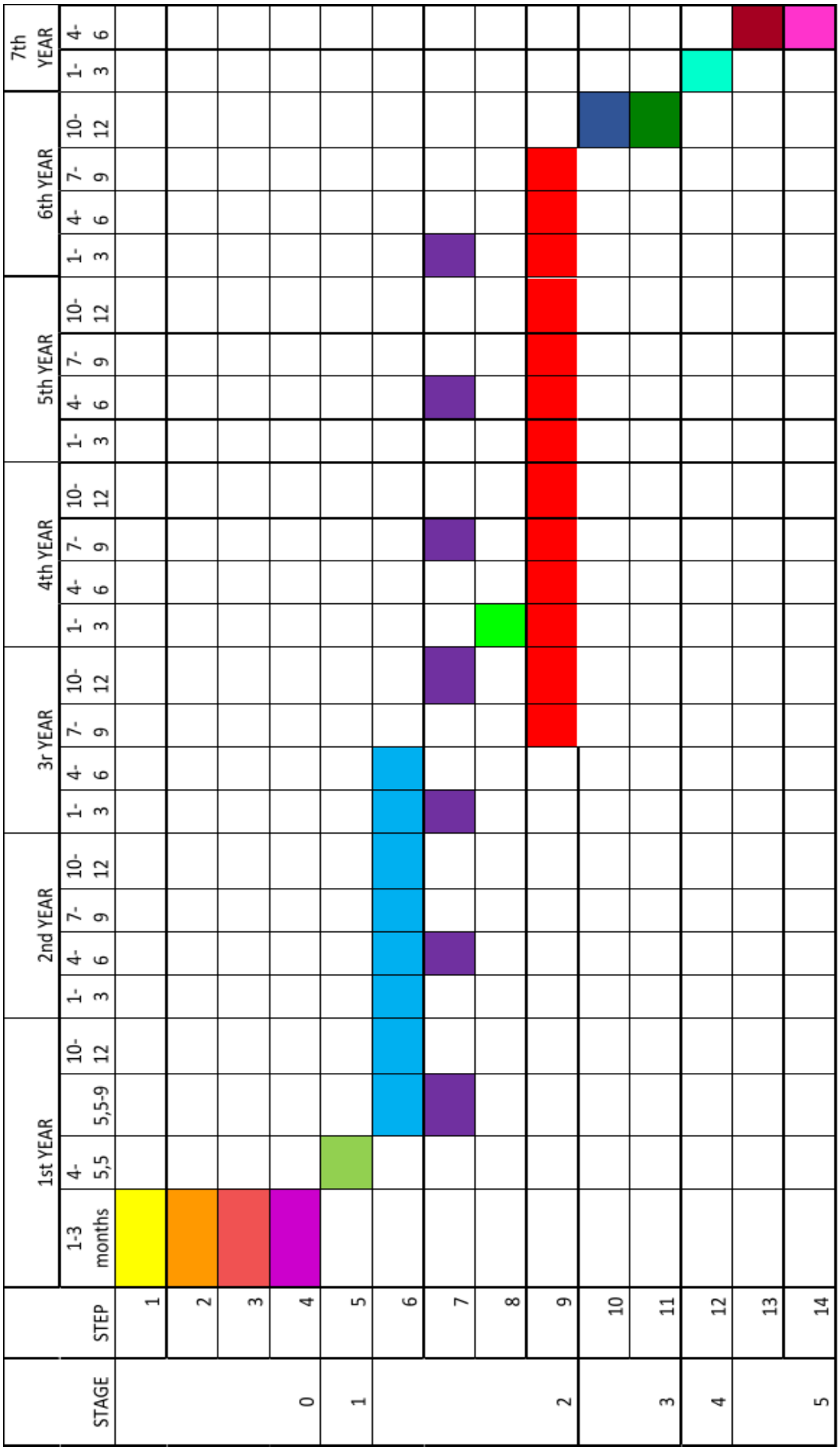
      They will write a final report with the most relevant conclusions of the study.

- **Stage 5: Redaction and results publication (3 months)**

    - **13th step**: The general coordinator will write a journal article of the study, explaining what has been done and the principal findings. Then, its publication will be asked for.

    - **14th step**: The general coordinator and one assistant elected by the all the research team, will attend to congresses to present the study nationally and internationally.

10.3. Chronogram



11. ETHICAL AND LEGAL ASPECTS

All basic ethical principles will be respected according the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human subjects (last revision in 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and by the Law 14/2007 of July 3rd of investigational project with invasive procedure. It will also be conducted with the fulfilment of the protocol, in accordance with ethical and methodological aspects of Good Clinical Practice guidelines in the European Union

The study must be evaluated by the Clinical Research Ethics Committee (CEIC) of all centres participating in the study and their approval must be obtained before initiating the study. ICO must also authorise the project execution.

Prior to the beginning of the investigation, every subject participating in the clinical trial must be properly informed about the study to the fullest extent using language and terms they are able to understand in order to allow a fully knowledgeable decision.

According to *Law 41/2002, de 14 de Noviembre, Básica reguladora de la autonomia del paciente y de derechos y obligaciones en materia de información y documentación clínica*, every single patient – or their relatives, in case a patient would not be able to be informed for him/herself - will be properly informed of the aim, procedures, anticipated benefits, and potential hazards of the study. Patients will be given a study information sheet (Annex 5) containing information about the study before they are included in the clinical trial. Prior to the participation in the clinical trial, the written informed consent (see Annex 6) must be obtained and signed by the patient or by the patient's legally acceptable representative and the investigator. A copy of the informed consent will be provided to the patient. It will also be explained to the participants that they are free to refuse entry into the study and to withdraw from the study at any time without prejudice to future treatment.

All the information and data collected from each patient during the course of the trial will be treated and used anonymously, preserving the confidentiality of the patient according to the *Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los derechos digitales*, the repealing Directive 95/46/EC (General Data Protection Regulation) and the European Parliament and Council regulation 2016/679 of April 27th in order to guarantee and protect the public liberties and fundamental rights of persons.

Subjects will be identified by their unique identification numeric code instead of their names. Personal patient data (personal identity and all personal medical information) will

be maintained in privacy. In any presentation of the results of this study at conferences or publications, the patient identities will remain confidential. The data access will be only available for the research team, the Ethical and Clinical Investigation Committee, the pertinent health authorities and those responsible for data analysis

All investigators will have to declare no conflict of interest in any of the aspects of the study.

12. STUDY LIMITATIONS

This study has limitations that have to be pointed:

- **Sample collection**

Using a non-probabilistic sampling method has an implicit risk of selecting a non-representative sample. Consecutive method has been chosen because is one of the non-probabilistic methods that induces less bias. Even so, sampling all patients and not discriminating any patient that enters in inclusion criteria parameters, we think that the bias is less probable.

- **Insufficient sample**

Although it is a multicentre prospective study with a long time for sample collection, we are aware that the sample may not seem very impressive but the calculated statistical power is surprisingly high (98%).

Regarding other retrospective studies with statistical significative results and the half of our sample we think the study has great potential.

- **Inclusion/exclusion criteria**

Including patients with no previous brain tumors could underestimate the number of secondary GBM studied and their effect as a co-variable in the final results, although the incidence of secondary GBM is very low.

- **Collaborating hospitals**

First of all, there is the probability that one or some of the hospitals reject participate in the study, which will decrease the size of the sample.

On the other hand, it is difficult to standardize a protocol for all of the three hospitals and to control if the protocol is being well executed. A lot of information collection bias may occur, to prevent it, meetings by videoconference (to minimize economic expenses) every 6 months will be done to assure a correct sample collection, correct clinical information collection and correct protocol development in general. Furthermore, to surely prevent this bias, project monitors (one for each hospital) could be hired to control the protocol's fulfilment.

- **Losses during follow-up**

The drop-out rate is expected to be of 5%. It is considered low, but that's because we think the patient will continue till the end for different reasons. First, the follow-up does

not interfere in the patient's national health system visits (as they are the same), so if they go to a medical revision, they will also be participating in the study follow-up. And second, no one, a part from the patient's physician will annoy him/her with questions or special requirements (unless if he/she does not come) that make them want to stand up for his/her right to abandon the study participation.

Aiming to decrease the follow up losses, a phone call will be made to patients and in default, to their relatives to know the reasons of their fault and to encourage them to come.

- **Evaluation of the variables**

There are possible information biases such as, may be not all the patient's dependent variables (IDH status and MGMT promoter methylation) will be possible to analyse. That's because not all GBM are biopsied or surgically resected as it depends on the patient's general clinical state, among other (location of the tumor, concomitant diseases, high surgical risk…) that are not contemplated in this study. Although in the exclusion criteria those patients that have diseases that contraindicate surgery at diagnosis, in the evolution of GBM can appear other causes that make impossible the surgery.

It could also happen that there is a biopsy made but with insufficient tissue sample to analyse the molecular information.

It is expected a low proportion of patients presenting this situation because in almost all cases at least a biopsy is performed.

- **Investigator variability**

As it is a multicentre study it is difficult to control all the data collected and make sure each physician is following the same template for evaluating the patients or to decide if a worseness is either pseudoprogression, radionecrosis or real tumor recurrence. That's why international scales and this study protocol will be used to standardize all the variables, regardless if they are used in the hospital's daily routine in GBM management.

In regards to imaging analysis for the study a double-blind test is made to assure that there is no influence of the investigators depending on the demographical or clinical characteristics of the patient. Then, another investigator will check the concordance of the two previous investigators to prevent variability among researchers.

- **Duration**

The long duration of the study, an expected 7 years and 3 months, with 3 years of follow-up will make difficult to prevent follow-up losses, which has been tried to avoid by contacting the patient if there is lack of assistance. It is probable that in such an amount of time we lose even some researcher, maybe because of tiredness or maybe for personal reasons, if that's the case, new collaborators will be searched (making they sign a declaration of no participation in most of the study time, if it corresponds).

The financing will be achieved by research scholarship, with a prorogation at the half of the study, we think budget will not be a problem.

13. BUDGET

SERVICE	COST	QUANTITY	SUBTOTAL
Personal expenses	None		**-**
Project manager	15 €/h	400 hours	**6.000 €**
Execution expenses (contact with patients that don't assist to follow-up, calls, printing informed consent and other information for the patient)	50€ in printing 50€ in calls		**100 €**
Statistical analyst	35 €/h	120 hours	**4.200 €**
Travels and diets, meetings, conference and presentations	Travels + congress (hotels, flights, trains): 1200 €/congress x 2 people Meetings of the research team (total 6 reunions) International congress	1.200 € None 2.500 €	**3.700 €**
Article publication (revision, edition, graphic design and preparation for the digital metadata)	1000 € per publication 600 € for English revision		**1.600 €**
TOTAL			**15.600 €**

14. FEASIBILITY

This study will take place in 3 three level hospitals that already have all available means to conduct the study. The protocol study and treatment for brain tumors, specifically GBM, is well established in ICOs centres.

The number of expected patients participating in the study (240 patients in 3 different hospitals in 2 years) is affordable, in a logistically point of view. Each hospital would have to attend a patient each 9 days (assuming 225 effective days/year), which is a feasible number.

Data collection is easy to realize, since there are two key points:

- There is a well-structured database where all clinical information and imaging and anatomopathological studies of a patient are upload and any physician belonging to a hospital linked with it has access. This facilitates the collection of the relevant information for the study.
- The patient will undergo all the entire diagnostic test and periodic clinical revisions that we need to perform in this study in the public health system anyway. The only thing we need is to have access to the patient's information and diagnostic MRI images, which reduces a lot the budget.

We think the main obstacle in the execution of this protocol is its duration, which make it very expensive and laborious, even so, we believe in its technical feasibility and in research scholarships to make it possible.

15. IMPACT ON THE NATIONAL HEALTH SYSTEM

Glioblastoma is the most frequent primary malignant brain tumor and has the lowest survival of all brain tumors. GBM diagnosis is made through a biopsy of the tumor and histological study of the tissue samples. The prognostic, classification and, in some cases, the treatment, depend of IDH-1 status and MGMT promoter methylation. Sadly, in some hospitals like HJT, complete sequencing IDH gens and MGMT promoter methylation can't be done, and tissue samples have to be sent to other reference centres (HJT send them to Badalona's ICO centre), delaying approximately in three to four weeks the certain diagnosis of GBM and in consequence, delaying the treatment and worsening the prognosis.

If the hypothesis of this project is validated by the results, it would permit start thinking of changing the way molecular features of GBM are defined, avoiding biopsy and its complications and incorporate multiparametric MRI as the basic diagnostic tool necessary to begin treatment.

Thus, patients could benefit from a fastest, non-invasive and more cost-effective diagnostic test that enables to start the treatment as soon as possible with a certain diagnosis and surely improves prognosis as 3-4 weeks in the evolution of GBM can mark a turning point.

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17. ANNEXES

17.1. Annex 1 – Prognostic factors of GBM

Table 2. Prognostic factors of GBM adapted from (8,10–12,17,18,34,66)

Good prognostic factors	Bad prognostic factors
• Age < 50 • MMSE 27-30 • KPS > 70 • Good initial general state • Tumoral size < 42mm • Umifocal lesion • Frontal lobe • GTR >70% with residual volume < 5cm3 • IDH 1-2 status mutated • Superficial tumor location • PDGFRA mutation • TP53 • LOH 10q • Pseudoprogression • Macroscopic GTR • Concomitant RT/QT	• Age > 70 years old • Bad neurological state • Presence of necrosis • Degree of enhancement • Deep tumor location • Tumoral size > 4cm • IDH wild-type • MGMT not methylated • EGFR expression • Corticoid treatment (worst clinical state, which make patients not candidates to aggressive treatment) • Subtotal surgical resection • higher LD prevalence in tumor tissues • Concomitant diseases

17.2. Annex 2 – Survival curves of GBM

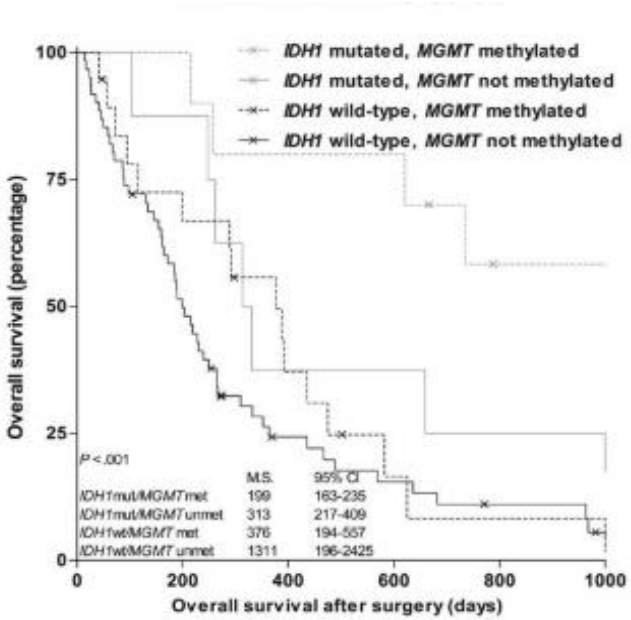


Figure 12. Survival curves of patients with IDH1 wild-type and unmethylated MGMT promoter; IDH1 wild-type and methylated MGMT promoter; IDH1 mutation and unmethylated MGMT promoter; IDH1 mutation and methylated MGMT promoter. P values were calculated by the log-rank test. Abbreviations: M.S., median survival From: Molenaar RJ . (66).

17.3. Annex 3 – Treatment algorithm of GBM

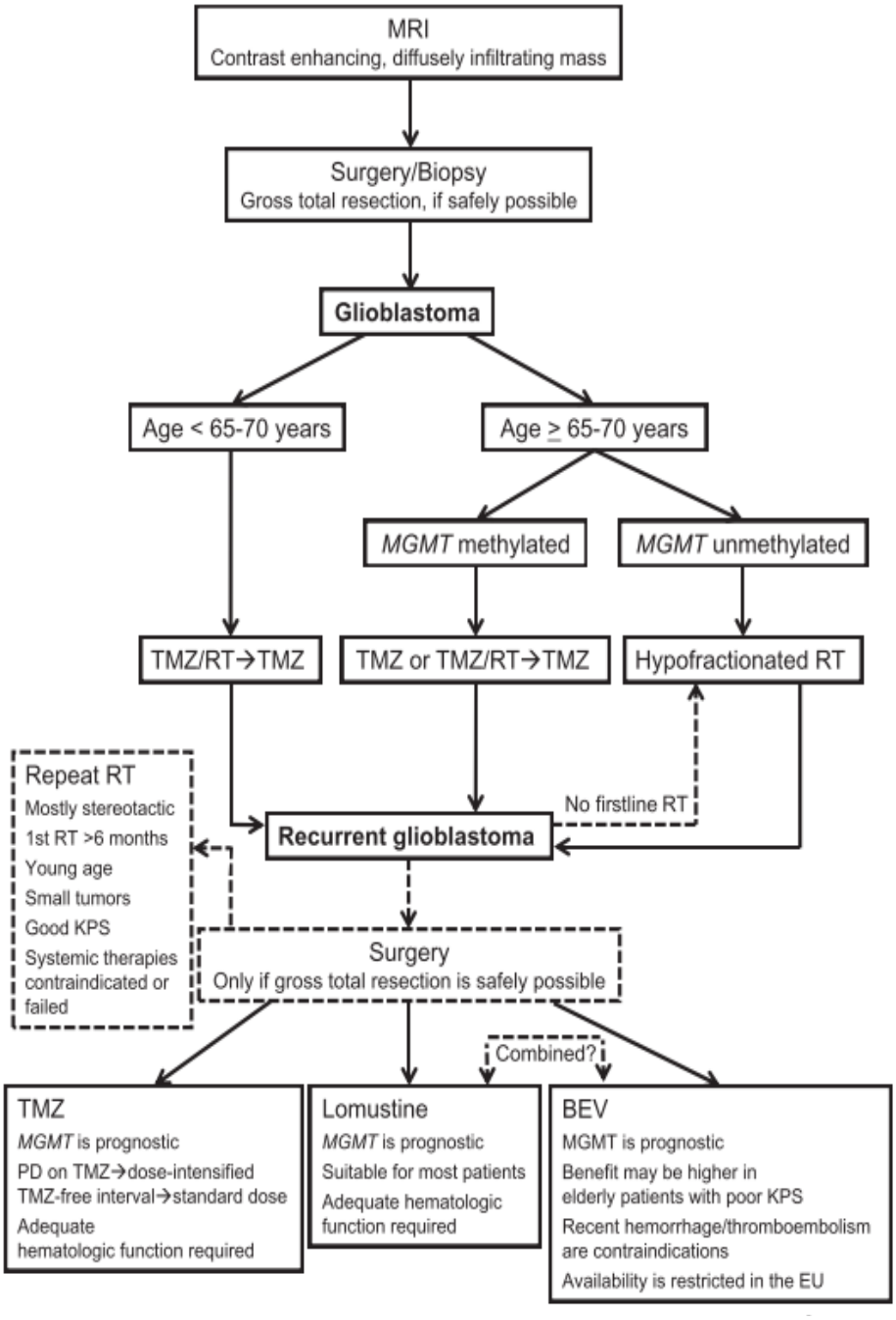


Figure 13. Therapeutic approach to glioblastoma. MRI, magnetic resonance imaging; MGMT, O-6-methylguanyl DNA methyltransferase gene promoter; TMZ, temozolomide 150–200 mg/m2 on 5/28 days; TMZ/RT, 30x2Gy=60 Gy with daily concomitant temozolomide at 75 mg/m2. From: (58)

17.4. Annex 4 – KPS, MMS and Barthel tests

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disable; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

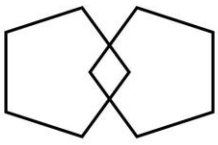
Figure14. KPS.

MINI MENTAL STATE EXAMINATION (MMSE)

Name:

DOB:

Hospital Number:

One point for each answer	DATE:		
ORIENTATION Year Season Month Date Time	...../ 5	...../ 5	...../ 5
Country Town District Hospital Ward/Floor	...../ 5	...../ 5	...../ 5
REGISTRATION Examiner names three objects (e.g. apple, table, penny) and asks the patient to repeat (1 point for each correct. THEN the patient learns the 3 names repeating until correct).	...../ 3	...../ 3	...../ 3
ATTENTION AND CALCULATION Subtract 7 from 100, then repeat from result. Continue five times: 100, 93, 86, 79, 65. (Alternative: spell "WORLD" backwards: DLROW).	...../ 5	...../ 5	...../ 5
RECALL Ask for the names of the three objects learned earlier.	...../ 3	...../ 3	...../ 3
LANGUAGE Name two objects (e.g. pen, watch).	...../ 2	...../ 2	...../ 2
Repeat "No ifs, ands, or buts".	...../ 1	...../ 1	...../ 1
Give a three-stage command. Score 1 for each stage. (e.g. "Place index finger of right hand on your nose and then on your left ear").	...../ 3	...../ 3	...../ 3
Ask the patient to read and obey a written command on a piece of paper. The written instruction is: "Close your eyes".	...../ 1	...../ 1	...../ 1
Ask the patient to write a sentence. Score 1 if it is sensible and has a subject and a verb.	...../ 1	...../ 1	...../ 1
COPYING: Ask the patient to copy a pair of intersecting pentagons 	...../ 1	...../ 1	...../ 1
TOTAL:	...../ 30	...../ 30	...../ 30

MMSE scoring
24-30: no cognitive impairment
18-23: mild cognitive impairment
0-17: severe cognitive impairment

OME Oxford Medical Education

Figure 15. MMS test.

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0–100):	_____

Figure 16. Barthel test

17.5. Annex 5 – Study information sheet

FULL INFORMACIÓ PER AL PACIENT

TÍTOL DE L'ESTUDI: _____

Investigador principal: _____

Centre: _____

Benvingut/a,

Ens posem en contacte amb vostè per invitar-la/lo a participar en un estudi d'investigació mèdica realitzat pel servei de Neuroradiologia de l'Hospital Dr. Josep Trueta de Girona.

Aquest estudi ha estat aprovat pel comitè ètic d'investigació clínica de tots els hospitals que hi participen així com per l'institut oncològic de Catalunya (ICO).

Primer de tot voldríem comentar-li que la seva participació és totalment voluntària i que en cas d'acceptar podria revocar-la en qualsevol moment sense conseqüències negatives ni canvis en la relació assistencial amb el seu doctor/a ni en el seu tractament i seguiment.

Descripció i objectiu de l'estudi

Aquest estudi va dirigit a persones que com vostè acaben de ser diagnosticades de Glioblastoma multiforme.

L'objectiu és identificar patrons radiològics que prediguin la presencia d'unes alteracions moleculars en el tumor.

La identificació per paràmetres de ressonància magnètica d'aquestes característiques moleculars ajudarien molt a un diagnòstic i tractament precoços, així com a una possible millora del pronòstic de persones que es trobaran en la mateixa situació que vostè.

Activitats de l'estudi

Si vostè accepta participar en aquest estudi les seves dades de seguiment i de proves complementaries seran analitzades per professionals especialitzats en el diagnòstic per la imatge de la seva malaltia. Les dades que fan referència a com es troba i que descriuen tan radiològicament com histològicament la seva malaltia quedaran registrades en una base de dades anònima pel seu anàlisis.

Vostè no haurà de participar activament en l'estudi ja que les dades s'extrauran del seu tracte assistencial, el mateix que es faria igualment encara que no participes en l'estudi.

Confidencialitat

Totes les dades mèdiques, com personals que ens proporcioni seran tractades amb la mes absoluta confidencialitat, d'acord amb la llei orgànica 3/2018, del 5 de desembre, de protecció de dades personals i garantia dels drets digitals, la directiva derogació 95/46 CE (reglament general de protecció de dades) i el reglament del parlament Europeu i del Consell 2016/679 del 27 d'Abril.

S'ha de remarcar que en cap cas la seva informació personal serà publica, i en cas que es publiquin els resultats en congressos o en publicacions científiques, sempre es farà de manera anònima i global, no individualitzada.

Compensació econòmica

Per participar en aquest estudi vostè no rebrà cap compensació econòmica, però tampoc li suposarà cap despesa. Els investigadors i col·laboradors de l'estudi tampoc rebran cap incentiu per la seva participació.

Contacte

Per qualsevol dubte o inquietud en relació a aquest estudi no dubti en posar-se en contacte amb:

Investigador principal: Dr./Dra: _____

Telèfon: _____

Correu electrònic: _____

HOJA DE INFORMACIÓN PARA EL PACIENTE

TÍTULO DEL ESTUDIO: _____

Investigador principal: _____

Centro: _____

Bienvenido/a,

Nos ponemos en contacto con usted para invitar-lo/la a participar en un estudio de investigación médica realizado por el Servicio de Neurorradiología del Hospital Dr. Josep Trueta de Girona.

Este estudio ha sido aprobado por el Comité ético de investigación clínica de todos los hospitales que participan, además del Instituto oncológico de Cataluña (ICO).

Antes de nada, nos gustaría comentarle que su participación es totalmente voluntaria y que, en caso de aceptar, podría revocarla en cualquier momento sin que ello tenga consecuencias negativas ni implique cambios en su relación asistencial con su doctor/a ni en el tratamiento i seguimiento que va a recibir.

Descripción i objetivo del estudio

Este estudio va dirigido a personas que como usted acaban de ser diagnosticadas de Glioblastoma multiforme. El objetivo es identificar patrones radiológicos que predigan la presencia de unas alteraciones moleculares en el tumor. La identificación por parámetros de resonancia magnética de estas características moleculares ayudaría mucho a un diagnóstico y tratamiento precoces, así como a una posible mejoría del pronóstico de personas que se encontraran en la misma situación que usted.

Actividades del estudio

Si usted acepta participar en este estudio sus datos del seguimiento y de pruebas complementarias serán analizados por profesionales especializados en el diagnóstico por la imagen de su enfermedad. Los datos que hacen referencia a como se encuentra i que describen tanto radiológicamente como histológicamente su enfermedad, quedaran registrados en una base de datos anónima para su análisis.

Usted no tendrá que participar activamente en el estudio, ya que sus datos se extraerán de su trato asistencial, lo mismo que haría si usted no participara en el estudio.

Confidencialidad

Todos los datos médicos, como personales que nos proporcione serán tratados con la más absoluta confidencialidad, de acuerdo con la ley orgánica 3/2018, del 5 de diciembre, de protección de datos personales i garantía de derechos digitales, la directiva derogación 95/46 CE (reglamento general de protección de datos) i el reglamento del parlamento europeo y del Consejo 2016/679 del 27 de abril.

Tenemos que remarcar que en ningún caso su información será publica, i en caso que se publiquen los resultados en congresos o publicaciones científicas, siempre se hará de forma anónima i global, nunca individualizada.

Compensación económica

Por participar en este estudio usted no recibirá ninguna compensación económica, pero tampoco le supondrá ningún gasto. Los investigadores i colaboradores del estudio tampoco recibirán ningún incentivo por su participación.

Contacto

Si tiene cualquier duda o inquietud en relación a este estudio no dude en ponerse en contacto con:

Investigador principal: Dr./Dra. _____

Telefono: _____

Correo electrónico: _____

17.6. Annex 6 - Informed consent sheet

CONSENTIMENT INFORMAT

Títol de l'estudi:

Jo, _____, amb DNI/NIF _____ accepto voluntàriament participar en aquest estudi i manifesto:

- Que he estat degudament informat/da per el dr./dra. _____ havent entès tota la informació que he pogut llegir del full d'informació i que m'han explicat, havent contat amb la opció de realitzar totes preguntes que he cregut convenients.
- Que puc negar-me a participar en aquest estudi sense la necessitat de donar explicacions i sense conseqüències en el meu tracte mèdic.
- Que les dades que doni seran utilitzades únicament amb finalitat de recerca mèdica, que seran completament confidencials i d'acord amb les lleis vigents.
- Que la meva participació es totalment voluntària i que podré revocar el consentiment prèviament firmat en qualsevol moment sense que això comporti cap conseqüència sobre el meu tractament i servei assistencial.

Firma del pacient Firma responsable/investigador/a

Lloc i data: _____, _____ de _____ del 20____.

REVOCACIÓ DEL CONSENTIMENT INFORMAT

Jo, _____, revoco el consentiment informat prèviament firmat per la participació en l'estudi especificat en aquest mateix document.

Firma del pacient Firma responsable/investigador/a

Lloc i data: _____, _____ de _____ del 20____.

CONSENTIMIENTO INFORMADO

Título del estudio:

Yo, _____, con DNI/NIF _____ acepto voluntariamente participar en este estudio y manifiesto:

- Que he estado debidamente informado/a por el Dr./Dra. _____ habiendo entendido toda la información que he podido leer en la hoja de información i que me han explicado, habiendo contado con la opción de realizar todas las preguntas que he creído convenientes.
- Que puedo negarme a participar en este estudio sin la necesidad de dar explicaciones y sin que ello tenga consecuencias en mi trato médico.
- Que los daros que dé serán utilizados únicamente con finalidad de investigación médica, que serán completamente confidenciales y de acuerdo con las leyes vigentes.
- Que mi participación es totalmente voluntaria y que podre revocar el consentimiento previamente firmado en cualquier momento sin que esto conlleve ninguna consecuencia negativa a mi tratamiento ni servicio asistencial.

Firma del paciente Firma del responsable/investigador/a

Lugar y fecha: _____, _____ de _____ del 20____.

REVOCACIÓN DEL CONSENTIMIENTO INFORMADO

Yo, _____, revoco el consentimiento informado previamente firmado para la participación en este estudio especificado en este mismo documento.

Firma del paciente Firma del responsable/investigador/a

Lugar y fecha: _____, _____ de _____ del 20____.

17.7. Annex 7 - Data collection form

ICO Institut Català d'Oncologia

Formulari recol·lecció dades

DATA ____ ____ ____
MM DD AA

HOSPITAL: ____

CODI PACIENT					
EDAT					
SEXE	H		D		
Nº Història clínica					
DATA DIAGNÒSTIC	__/__/____				
TELÈFON CONTACTE personal					
TELÈFON CONTACTE familiar	Número de telèfon				
ADREÇA DOMOCILI personal	Direcció		Ciutat		
	Provincia		Codi postal		
ADREÇA DOMOCILI personal	Direcció		Ciutat		
	Provincia		Codi postal		
Índex KARNOFSKY					
MME					
Test de Barthel					
Tumors SNC previs	SÍ				
Malalties SNC prèvies	SÍ	NO	QUINA: _____		
Símptomes neurològics:	SÍ	NO			
- *Signes hipertensió intracranial (algun: cefalea/vòmits/diplopia):*	SÍ	NO			
- *Focalitat neurològica:*	SÍ	NO			
- *Atacs epilèptics:*	SÍ	NO			

ICO Institut Català d'Oncologia

Formulari recol·lecció dades

MUTACIÓ IDH	SÍ (MUTAT)	NO (WILD-TYPE)	
METILACIÓ PROMOTOR MGMT	SÍ (METILAT)	NO (NO METILAT)	
TRACTAMENT REBUT	Cirurgia		
	RT (esquema i dosis)		
	QT (tipus i dosis)		
	Glucocorticoides (dosi i tipus)		
APARICIÓ PSEUDOPROGRESSIÓ RADIOLÒGICA	SÍ	NO	NO AVALUABLE
APARICIÓ RADIONECROSIS	SÍ	NO	NO AVALUABLE
Data RECURRENCIA	__/__/____		
Data EXITUS	__/__/____		
OBSERVACIONS			

Pe qualsevol dubte a l'hora d'omplir formulari

CONTACTAR AMB INVESTIGADOR PRINCIPAL: *Dr.Dra.* _____

Telèfon.:

Correu electrònic: